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regeneration medium comprises 5-BrIAA, zeatin riboside and ABA. In some embodiments, the plant tissue sample may be derived from a mature plant tissue. Suitable plants include, but are not limited to, maize, wheat, sorghum, sugar beets, potatoes, soy beans, rice and other plants commonly cultivated. In some embodiments, the plant sample may be derived from maize. In some embodiments, the plant sample may be a seed or a portion of a seed. In some embodiments, the plant sample may be derived from a maize seed. In some embodiments, the plant sample may a seed or a portion of a seed from a maize variety selected from a group consisting of B73, H99 and PA91. In other embodiments, the method may further comprise the step of incubating the plant tissue at a reduced temperature before excision of the sample. In some embodiments, a reduced temperature may be from about 0°C to about 20°C, preferably from about 0°C to about 10°C, more preferably from about 0°C to about 5°C, and most preferably about 4°C. In some embodiments, one or more of the steps are performed in membrane-based liquid culture.

The present invention also relates to kits for carrying out the methods of the invention, and particularly for use in generating a callus, preferably an embryogenic callus. In some preferred embodiments, the present invention may provide kits for the transformation and/or regeneration of plant samples. Such kits may include one or more containers, one or more medium formulations, solid supports such as membranes and/or agar. Such kits may optionally comprise one or more additional components selected from the group consisting of one or more suitable buffers, one or more cytokinins and one or more auxins.

Other preferred embodiments of the present invention will be apparent to one of ordinary skill in light of what is known in the art, in light of the following drawings and description of the invention, and in light of the claims.--

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#### REMARKS

The specification is amended to correct minor typographical errors by replacing the BRIEF SUMMARY OF THE INVENTION with a new BRIEF SUMMARY OF THE INVENTION section. In particular, the term "dicamba" was misspelled as "dicmamba." This error is corrected as a result

of this amendment. Support for the correct spelling is found in the as-filed specification at page 1, line 31. The amendments do not represent the addition of new material to the specification.

It is believed that this Preliminary Amendment does not require the payment of a fee. If this is incorrect, please deduct the appropriate fee from deposit account 07-1969.

Respectfully submitted,

A handwritten signature in black ink, appearing to be 'J. A. Baker', written over the typed name.

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MARKED-UP COPY OF CHANGES TO THE SPECIFICATION  
USSN 09/805,383

**BRIEF SUMMARY OF THE INVENTION**

A principal object of the present invention is to provide a growth affecting composition comprising one or more indole-3-acetic acid (IAA) derivatives. The compositions of the present invention play a significant role in inducing a number of growth-affecting responses in a variety of plant species. Suitable IAA derivatives are described in United States patent application serial number 08/758,416 entitled Auxinic Analogues of Indole-3-Acetic Acid, filed November 29, 1996, which is specifically incorporated herein by reference. In some preferred embodiments, the compositions of the present invention may comprise a substituted derivative of IAA. The derivatives of IAA of the present invention may comprise one or more substitutions in the IAA molecule. In some preferred embodiments, the IAA derivative may be a mono-substituted IAA molecule. In some preferred embodiments, the IAA derivative of the present invention may be a di-substituted IAA. In some preferred embodiments, the IAA derivative of the present invention may be a multi-substituted IAA molecule. The derivatives may be in the form of an acid, ester, amide or salt. In some preferred embodiments, the present invention contemplates growth affecting compositions comprising a mono-substituted IAA with a substituent group in the 2, 4, 5, 6, or 7 position of the IAA wherein the substituent may be a halogen, an alkyl group, an alkoxy group, an acyl group, an acylamido group or an acyloxy group having 1-10 carbons. In some preferred embodiments the IAA derivative may be a di-substituted IAA derivative with substituents on two of the 2, 4, 5, 6, or 7 positions of the IAA wherein the substituents may be the same or different and may be a halogen, an alkyl group, an alkoxy group, an acyl group, an acylamido group or an acyloxy group having 1-10 carbons. In some preferred embodiments the IAA derivative may be a multi-substituted IAA derivative with substituents on three or more of the 2, 4, 5, 6, or 7 positions of the IAA wherein the substituents may be the same or different and may be a halogen, an alkyl group, an alkoxy group, an acyl group, an acylamido group or an acyloxy group having 1-10 carbons. In some preferred embodiments, the compositions of the present invention may comprise the IAA derivative 5-bromoindole-3-acetic acid (5-BrIAA) in the form of an acid, ester, amide or salt in an amount sufficient to achieve a plant growth affecting response. The invention contemplates the use of 5-BrIAA to affect growth in both monocotyledonous and dicotyledonous plants.

It is also an object of the invention to provide a composition for affecting plant growth comprising one or more indole-3-acetic acid (IAA) derivatives in a mixture with one or more additional plant growth regulators, for example, an auxin, a cytokinin, a gibberellin, an abscisic acid, etc., in definite proportions and concentrations for wide application to various plants in order to achieve a plant growth affecting response. In one aspect, the composition may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof and may further comprise one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments, the composition may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and may further comprise one or more compounds selected from a group consisting of (2,4-Dichlorophenoxy)acetic acid (2, 4-D), 6-benzylaminopurine (BAP), abscisic acid (ABA), zeatin riboside, kinetin, (2-Isopentyl)adenine (2iP) and [dicmamba] dicamba. In some embodiments, the composition may comprise a mono-substituted IAA derivative and may further comprise at least one plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the composition may comprise 5-BrIAA and may further comprise at least one plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the composition may comprise 5-BrIAA and may further comprise at least one compound selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the composition may comprise 5-BrIAA, 2,4-D, BAP and ABA. In some embodiments, the composition may comprise 5-BrIAA, zeatin riboside and ABA. In specific embodiments, the invention was exemplified with compositions comprising 5-BrIAA and a cytokinin to affect the growth of plants.

In one aspect, the present invention provides a composition formed by mixing one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof with one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one

or more abscisic acids and mixtures thereof, etc. In some embodiments, the composition may be formed by mixing one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives with one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the composition can be formed by mixing a mono-substituted IAA with a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the composition can be formed by mixing 5-BrIAA with a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the composition can be formed by mixing 5-BrIAA with a plant growth regulating compound selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the composition is formed by mixing 5-BrIAA, 2,4-D, BAP and ABA. In some embodiments, the composition may be formed by mixing 5-BrIAA, zeatin riboside and ABA.

It is a further object of the invention to provide a culture medium for affecting plant growth comprising a mixture of one or more indole-3-acetic acid (IAA) derivatives and one or more additional plant growth regulators (e.g. one or more auxins, cytokinins, giberellins and/or abscisic acids) as components of medium which sustains the plant during plant development or tissue regeneration and also serves as a vehicle whereby the one or more indole-3-acetic acid (IAA) derivatives may be applied. In one aspect, the medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof and may further comprise one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments, the medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and may further comprise one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the medium may comprise a mono-substituted IAA derivative and may further comprise at least one plant growth regulating

compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the medium may comprise 5-BrIAA and may further comprise at least one plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the medium may comprise 5-BrIAA and may further comprise at least one compound selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the medium may comprise 5-BrIAA, 2,4-D, BAP and ABA. In some embodiments, the medium may comprise 5-BrIAA, zeatin riboside and ABA. In specific embodiments, the invention was exemplified with compositions comprising 5-BrIAA and a cytokinin to affect the growth of plants.

It is an object of the present invention to provide a medium for the formation of a callus, preferably, an embryogenic callus, from a plant sample. In some embodiments, the callus formation medium may comprise a callus inducing amount of one or more plant growth regulating compounds selected from a group consisting of auxins, cytokinins, gibberellins and abscisic acids. In some preferred embodiments, the callus formation medium comprises one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof and further comprises one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments, the callus formation medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and may further comprise one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the callus formation medium comprises 5-BrIAA, 2,4-D, BAP and ABA. In some embodiments, the medium may comprise 5-

BrIAA.

It is an object of the present invention to provide a medium for the regeneration of a plant sample. In some embodiments, the plant sample may be a callus, preferably an embryogenic callus. In some embodiments, the medium may comprise a regeneration inducing amount of one or more plant hormones selected from a group consisting of auxins, cytokinins, gibberellins and abscisic acids. In some preferred embodiments, the regeneration medium comprises one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof, and further comprises one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments of the present invention, the regeneration medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and mixtures thereof, and may further comprise one or more compounds selected from a group consisting of 2,4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicamba] dicamba. In some embodiments, the regeneration medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the regeneration medium comprises 5-BrIAA, zeatin riboside and ABA. It is an additional object of the invention to provide a method of affecting plant growth which comprises the step of applying to a plant sample an effective amount of a plant growth-affecting composition comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives, and/or one or more multi-substituted IAA derivatives or mixtures thereof, and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments of the present invention, the composition may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and mixtures thereof, and may further comprise one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicamba] dicamba. In some embodiments, the composition comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or

more gibberellins, one or more abscisic acids and mixtures thereof. In specific embodiments, the invention was exemplified with compositions comprising 5-BrIAA and a cytokinin to affect the growth of plants. In some embodiments, the method may further comprise a step of incubating the plant sample in the presence of a plant growth- affecting composition. In some embodiments, the plant sample may be an entire plant, a plant locus, a plant cell, a plant tissue, a plant seed or a portion of any of these. In some preferred embodiments, the plant sample is all or a portion of a transgenic plant.

It is an object of the present invention to provide a method of regenerating a plant from a plant sample, comprising the steps of providing a sample from a plant, culturing the sample in contact with a regeneration medium under conditions causing the regeneration of the plant sample, the regeneration medium comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof, and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments of the present invention, the regeneration medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and may further comprise one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the regeneration medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the regeneration medium comprises 5-BrIAA, zeatin riboside and ABA. In some embodiments, the plant tissue sample may be derived from a mature plant tissue. Suitable plants include, but are not limited to, maize, wheat, sorghum, sugar beets, potatoes, soy beans, rice and other plants commonly cultivated as food sources. In some embodiments, the plant tissue is derived from a mature maize seed. In other embodiments, the method may further comprise the step of incubating the plant at a reduced temperature before excision of the sample. In some embodiments, the culturing step is performed in membrane-based liquid culture.



It is an object of the present invention to provide a method of regenerating a plant from a differentiated plant tissue, comprising the steps of providing a sample from a plant, the sample comprising differentiated plant tissue, culturing the sample in contact with a callus formation medium under conditions causing the formation of a callus, the callus formation medium comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof, and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc., and regenerating a plant from the callus. In some embodiments, the callus formation medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and may further comprise one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the callus formation medium comprises 5-BrIAA, 2,4-D, BAP and ABA. In some embodiments, the method may further comprise the step of transferring the callus to a regeneration medium under conditions causing the regeneration of the callus, the regeneration medium comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments of the present invention, the regeneration medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and mixtures thereof and may further comprise one or more compounds selected from a group consisting of 2,4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the regeneration medium comprises 5-BrIAA and a plant growth regulating compound selected from

a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the regeneration medium comprises 5-BrIAA, zeatin riboside and ABA. In some embodiments, the callus formation medium is different from the regeneration medium. In some embodiments, the plant sample may be derived from a mature plant tissue. Suitable plants include, but are not limited to, maize, wheat, sorghum, sugar beets, potatoes, soy beans, rice and other plants commonly cultivated. In some embodiments, the plant sample may comprise all or a portion of a mature maize seed. In some embodiments, the method may comprise the additional step of amplifying the callus before transferring the callus to the regeneration medium. In other embodiments, the method may further comprise the step of incubating the plant tissue at a reduced temperature before excision of the sample. In some embodiments, a reduced temperature may be from about 0°C to about 20°C, preferably from about 0°C to about 10°C, more preferably from about 0°C to about 5°C, and most preferably about 4°C. In some embodiments, the culturing step is performed in membrane-based liquid culture.

It is an object of the present invention to provide a method for the production of an embryogenic callus from a plant sample. In some embodiments, the method may comprise providing a plant sample, contacting the plant sample with a composition comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof, and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc., and culturing the sample under conditions causing the formation of an embryogenic callus. In some embodiments, the composition may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and may further comprise one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the composition comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the composition comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the composition comprises 5-BrIAA, 2,4-D, BAP and

ABA. In some preferred embodiments, the composition may comprise 5-BrIAA. In some embodiments, the sample may be derived from a mature plant. Suitable plants include, but are not limited to, maize, wheat, sorghum, sugar beets, potatoes, soy beans, rice and other plants commonly cultivated. In some embodiments, the plant sample may be derived from maize. In some embodiments, the sample may be a seed or a portion of a seed. In some embodiments, the plant sample may be derived from a maize seed. In some embodiments, the plant sample may be a seed or a portion of a seed from a maize variety selected from a group consisting of B73, H99 and PA91.

It is an object of the present invention to provide a method for the production of an embryogenic callus from a plant sample wherein the method comprises providing a plant sample, incubating the sample at a reduced temperature and culturing the plant sample in the presence of a callus formation medium, the callus formation medium comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc., and regenerating a plant from the callus. In some embodiments, the callus formation medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and may further comprise one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicamba] dicamba. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicamba] dicamba. In some embodiments, the callus formation medium comprises 5-BrIAA, 2,4-D, BAP and ABA. In some preferred embodiments, the medium may comprise 5-BrIAA. In some embodiments, a portion of the sample may be excised and cultured after the sample has been incubated at a reduced temperature. In some embodiments, a reduced temperature may be from about 0°C to about 20°C, preferably from about 0°C to about 10°C, more preferably from about 0°C to about 5°C, and most preferably about 4°C. In some embodiments, the plant sample may be derived from a mature plant.

Suitable plants include, but are not limited to, maize, wheat, sorghum, sugar beets, potatoes, soy beans, rice and other plants commonly cultivated. In some embodiments, the plant sample may be derived from maize. In some embodiments, the plant sample may be a seed or a portion of a seed. In some embodiments, the plant sample may be derived from a maize seed. In some embodiments, the plant sample may be derived from a maize variety selected from a group consisting of B73, H99 and PA91. In some embodiments, the incubation step may be performed at 4 °C for from about 1 day to about 10 days. In some embodiments, the incubation step may be performed for 4 days.

It is an object of the present invention to provide a method of regenerating a shoot from a callus, comprising the steps of contacting a callus with a regeneration medium and incubating the callus under conditions causing the regeneration of a shoot from the callus. In some embodiments, the medium may comprise a regeneration-inducing amount of one or more plant hormones selected from a group consisting of auxins, cytokinins, gibberellins and abscisic acids. In some preferred embodiments, the regeneration medium comprises one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof, and further comprises one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments of the present invention, the regeneration medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and mixtures thereof and may further comprise one or more compounds selected from a group consisting of 2,4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicamba] dicamba. In some embodiments, the regeneration medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the regeneration medium comprises 5-BrIAA, zeatin riboside and ABA. In some embodiments, the callus may be derived from a mature plant. Suitable plants include, but are not limited to, maize, wheat, sorghum, sugar beets, potatoes, soy beans, rice and other plants commonly cultivated. In some embodiments, the callus may be derived from maize. In some embodiments, the callus may be derived from a seed or a portion of a seed. In some embodiments, the callus may be derived from a maize seed. In some

embodiments, the callus may be derived from a from a maize variety selected from a group consisting of B73, H99 and PA91.

It is an object of the present invention to provide a method for the regeneration of a transformed plant, comprising the steps of providing a plant sample, culturing the plant sample in the presence of a callus formation medium, the callus formation medium comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof, and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc., to produce an embryogenic callus, transforming the callus and incubating the transformed callus under conditions causing the regeneration of the callus. In some embodiments, the callus formation medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and may further comprise one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicmamba. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicmamba. In some embodiments, the callus formation medium comprises 5-BrIAA, 2,4-D, BAP and ABA. In some embodiments, the method may further comprise the step of transferring the callus to a regeneration medium under conditions causing the regeneration of the callus, the regeneration medium comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments of the present invention, the regeneration medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and mixtures thereof and may further comprise one or more compounds selected from a group consisting of 2,4-D, BAP, ABA,

zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the regeneration medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the regeneration medium comprises 5-BrIAA, zeatin riboside and ABA. In some embodiments, the callus formation medium is different from the regeneration medium. In some embodiments, the plant tissue sample may be derived from a mature plant tissue. Suitable plants include, but are not limited to, maize, wheat, sorghum, sugar beets, potatoes, soy beans, rice and other plants commonly cultivated. In some embodiments, the plant sample may be derived from maize. In some embodiments, the plant sample may be a seed or a portion of a seed. In some embodiments, the plant sample may be derived from a maize seed. In some embodiments, the plant sample may be a seed or a portion of a seed from a maize variety selected from a group consisting of B73, H99 and PA91. In some embodiments, the method may comprise the additional step of amplifying the callus before transferring the callus to the regeneration medium. In other embodiments, the method may further comprise the step of incubating the plant tissue at a reduced temperature before excision of the sample. In some embodiments, a reduced temperature may be from about 0°C to about 20°C, preferably from about 0°C to about 10°C, more preferably from about 0°C to about 5°C, and most preferably about 4°C. In some embodiments, one or more of the steps are performed in membrane-based liquid culture.

It is an object of the present invention to provide a method for the regeneration of a transformed plant, comprising the steps of providing a plant sample, transforming the plant sample and culturing the plant sample in the presence of a callus formation medium to produce a transformed callus, the callus formation medium comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. and incubating the transformed callus under conditions causing the regeneration of the callus. In some embodiments, the callus formation medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and may further comprise one or more compounds selected from a group consisting of 2, 4-D, BAP, ABA, zeatin

riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the callus formation medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of 2, 4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the callus formation medium comprises 5-BrIAA, 2,4-D, BAP and ABA. In some embodiments, the method may further comprise the step of transferring the callus to a regeneration medium under conditions causing the regeneration the callus, the regeneration medium comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments of the present invention, the regeneration medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and mixtures thereof and may further comprise one or more compounds selected from a group consisting of 2,4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicmamba] dicamba. In some embodiments, the regeneration medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the regeneration medium comprises 5-BrIAA, zeatin riboside and ABA. In some embodiments, the callus formation medium is different from the regeneration medium. In some embodiments, the plant tissue sample may be derived from a mature plant tissue. Suitable plants include, but are not limited to, maize, wheat, sorghum, sugar beets, potatoes, soy beans, rice and other plants commonly cultivated. In some embodiments, the plant sample may be derived from maize. In some embodiments, the plant sample may be a seed or a portion of a seed. In some embodiments, the plant sample may be derived from a maize seed. In some embodiments, the plant sample may a seed or a portion of a seed from a maize variety selected from a group consisting of B73, H99 and PA91. In some embodiments, the method may comprise the additional step of amplifying the callus before transferring the callus to the regeneration medium. In other embodiments, the method may further comprise the step of

incubating the plant tissue at a reduced temperature before excision of the sample. In some embodiments, a reduced temperature may be from about 0°C to about 20°C, preferably from about 0°C to about 10°C, more preferably from about 0°C to about 5°C, and most preferably about 4°C. In some embodiments, one or more of the steps are performed in membrane-based liquid culture.

It is an object of the present invention to provide a method for the regeneration of a transformed plant, comprising the steps of providing a plant sample, transforming the plant sample and culturing the plant sample in the presence of a regeneration medium comprising one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives or mixtures thereof and further comprising one or more additional plant growth regulators, for example, one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof, etc. In some embodiments of the present invention, the regeneration medium may comprise one or more mono-substituted IAA derivatives and/or one or more di-substituted IAA derivatives and/or one or more multi-substituted IAA derivatives and mixtures thereof and may further comprise one or more compounds selected from a group consisting of 2,4-D, BAP, ABA, zeatin riboside, kinetin, 2iP and [dicamba] dicamba. In some embodiments, the regeneration medium comprises 5-BrIAA and a plant growth regulating compound selected from a group consisting of one or more auxins, one or more cytokinins, one or more gibberellins, one or more abscisic acids and mixtures thereof. In some embodiments, the regeneration medium comprises 5-BrIAA, zeatin riboside and ABA. In some embodiments, the plant tissue sample may be derived from a mature plant tissue. Suitable plants include, but are not limited to, maize, wheat, sorghum, sugar beets, potatoes, soy beans, rice and other plants commonly cultivated. In some embodiments, the plant sample may be derived from maize. In some embodiments, the plant sample may be a seed or a portion of a seed. In some embodiments, the plant sample may be derived from a maize seed. In some embodiments, the plant sample may be a seed or a portion of a seed from a maize variety selected from a group consisting of B73, H99 and PA91. In other embodiments, the method may further comprise the step of incubating the plant tissue at a reduced temperature before excision of the sample. In some embodiments, a reduced temperature may be from about 0°C to about 20°C, preferably from about 0°C to about 10°C, more preferably from about 0°C to about 5°C, and most preferably about 4°C. In some embodiments, one or more of the steps are performed in membrane-based liquid culture.



The present invention also relates to kits for carrying out the methods of the invention, and particularly for use in generating a callus, preferably an embryogenic callus. In some preferred embodiments, the present invention may provide kits for the transformation and/or regeneration of plant samples. Such kits may include one or more containers, one or more medium formulations, solid supports such as membranes and/or agar. Such kits may optionally comprise one or more additional components selected from the group consisting of one or more suitable buffers, one or more cytokinins and one or more auxins.

Other preferred embodiments of the present invention will be apparent to one of ordinary skill in light of what is known in the art, in light of the following drawings and description of the invention, and in light of the claims.